# Why FRET about Ran?

**The Ran GTPase drives nucleocytoplasmic transport, stabilizes mitotic spindles, and catalyzes nuclear envelope formation. A unifying explanation of these functions is that RanGTP produces an organizing field or "atmosphere" around chromatin and acts as a spatial marker. This RanGTP field has now been visualized using fluorescent biosensors.**

The concepts of morphogen gradients and morphogenetic field have been staples of experimental embryology for many decades. However, the idea that a parallel organizing principle may act within the cell has not been widely considered. Clearly, the localized activation of signaling pathways and transient changes in ion concentrations within the cellular environment do play fundamental roles in cell organization (Jin et al., 2000; Kraynov et al., 2000; Lauffenburger and Horwitz, 1996). However, evidence has been lacking that stable gradients of such species exist and regulate structural elements of the cell. Now Kalub and colleagues (2002) have visualized a RanGTP gradient that is generated by chromatin in frog egg extracts. This gradient is thought to be required not only during interphase cells to define the nuclear compartment (Gorlich and Kutay, 1999), but also during mitosis to guide spindle microtubules (Karsenti and Vernos, 2001), and after mitosis to target nuclear envelope formation (Hetzer et al., 2000). These diverse functions can be reconciled by the notion that the RanGTP gradient marks chromatin location within the cell and behaves as a classic example of an intracellular organizing field: it is kinetically dynamic, but temporally stable, and it provides a spatial cue that guides morphological changes.

The Ran GTPase is an essential protein for the eukaryotic cell and like other GTPases is regulated by a guanine nucleotide exchange factor (RanGEF, also called RCC1), which produces RanGTP, and by a GTPase activating protein (RanGAP), which converts the RanGTP to RanGDP (Gorlich and Kutay, 1999). The organizing field is generated by the asymmetric distribution of these two factors: RanGAP is largely soluble, while RCC1 is tightly associated with chromatin, through histones H2A/2B. This simple arrangement should, in the presence of free GTP, produce an "atmosphere" of RanGTP at the chromatin surface (see Figure, panel [A]).

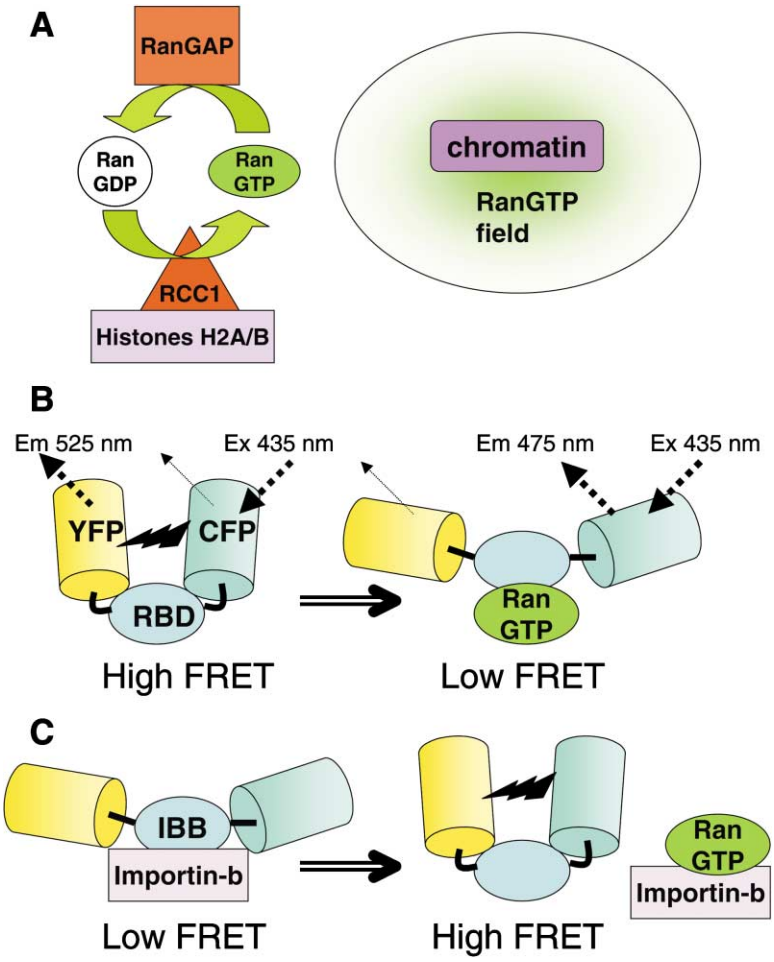
To visualize RanGTP, Kalub and colleagues used a Ran binding domain from the budding yeast protein, Yrb1p, to which they attached a yellow fluorescent protein (YFP) at the N terminus and a cyan fluorescent protein (CFP) at the C terminus. This construct (YRC) does not bind RanGDP but has a high affinity for RanGTP. The interaction with RanGTP pushes the N and C termini apart, increasing the CFP-YFP separation (see Figure, panel [B]). The emission spectrum of CFP has substantial overlap with the absorption spectrum of YFP, so that radiationless energy transfer can occur between the two when CFP is excited. The efficiency of this transfer varies with the sixth power of the distance between the donor and acceptor, thus providing a very sensitive sensor for conformational changes. So in the absence of RanGTP, the fluorescence resonance energy transfer (FRET) signal of the YRC sensor is large, and it is decreased by RanGTP binding.

To detect a RanGTP gradient, demembranated sperm nuclei were added to a frog mitotic egg extract. The sperm chromatin induces the formation of bipolar spindles, with chromosomes positioned at the metaphase plate. When this reaction was performed in the presence of the YRC sensor, a reduced FRET signal was observed in the immediate vicinity of the chromosomes, indicating the production of RanGTP in this neighborhood. Importantly, the change in FRET was abolished by the addition of a dominant interfering mutant of Ran (T24N), which inhibits RanGEF function.

There are many potential artifacts in FRET microscopy, especially those that might quench the fluorescence signal. Kalab and coworkers therefore made a second biosensor that produces the opposite signal in the presence of RanGTP. This probe takes advantage of a fragment of the import cargo receptor, importin-$\alpha$, called IBB. The IBB domain binds to a karyopherin called importin-$\beta$ (which can translocate rapidly through the nuclear pores). RanGTP, but not RanGDP, displaces IBB from importin-$\beta$. Importantly, the free IBB domain is unstructured, so the N and C termini can approach closely to one another, but, when complexed with importin-$\beta$, it is locked into a rigid $\alpha$-helical conformation (Cingolani et al., 1999). Therefore, a YFP-IBB-CFP construct (called YIC) should produce a low FRET signal in the presence of importin-$\beta$, unless RanGTP is present, when the YIC will be released from the importin-$\beta$, and the FRET signal will increase (see Figure, panel [C]). This phenomenon was observed both in vitro, using recombinant proteins, and in mitotic egg extracts containing assembled spindles. The YIC FRET signal was strongest in the immediate vicinity of the chromosomes and was ablated by the addition of the T24N Ran mutant.

The vectoriality of nucleocytoplasmic transport is believed to be driven by a large RanGTP gradient across the nuclear pores, which computer modeling has predicted to be about 500-fold for free RanGTP (Smith et al., 2002). The YRC sensor was employed to test this hypothesis, using interphase nuclei generated in the egg extracts. Although, as expected, the probe accumulated in the nucleus, the nuclear FRET signal was very low, suggesting a concentration difference between free nuclear and cytoplasmic RanGTP of at least 200-fold. This strong agreement with the model helps validate both the computational approach and the use of the YRC probe as a RanGTP detector.

One value of the new FRET probes described by Kalab et al. is that they can now be used, perhaps in conjunction computational modeling, to help decipher the controls that may modulate the RanGTP field during mitosis. For example, Virtual Cell simulations (Smith et al., 2002) could be used to model the field using known values for the rate and diffusion constants of the RanGEF and RanGAP. Such models may reveal whether additional factors (activation of RanGEF, or Ran binding to the chromatin, for instance) need to be considered to account for the extent of the field visualized by the FRET




Visualizing Ran Gradients in the Nucleus

(A) Generation of the RanGTP field. RanGTP is generated at the surface of chromatin by RanGEF, which catalyzes guanine nucleotide exchange on Ran. The RanGEF binds with high affinity to histones H2A/B. RanGTP is destroyed in the cytoplasm by soluble RanGAP, which catalyzes the hydrolysis of the GTP on Ran.

(B) Design of the YRC biosensor used by Kalab et al. RBD is the Ran binding domain of the yeast protein Yrb1p. In the absence of RanGTP, the fluorophores, CFP and YFP, are sufficiently close that radiationless energy transfer to the YFP is efficient. Binding of RanGTP moves the fluorophores apart, reducing FRET efficiency.

(C) Design of the YIC biosensor. IBB is the N-terminal domain of importin-$\alpha$, and binds with high affinity to the nuclear transport receptor, importin-$\beta$, which holds the N and C termini of IBB apart. RanGTP triggers the dissociation of IBB from importin-$\beta$, allowing its N and C termini to approach one another. The CFP and YFP are therefore brought into proximity, and FRET increases.

probes. The general approach of using FRET biosensors may also reveal that many other proteins form polarized organizing fields within the cell to control migration, spindle orientation, differentiation, and other processes that are spatially asymmetric. The ease with which such sensors can now be made and used will ensure rapid progress in this area.

**Ian G. Macara**
Center for Cell Signaling, and Department of Pharmacology
Box 800577, HSC
University of Virginia
Charlottesville, Virginia 22908

**Selected Reading**

Cingolani, G., Petosa, C., Weis, K., and Muller, C.W. (1999). Nature *399*, 221–229.

Gorlich, D., and Kutay, U. (1999). Annu. Rev. Cell Dev. Biol. *15*, 607–660.

Hetzer, M., Bilbao-Cortes, D., Walther, T.C., Gruss, O.J., and Mattaj, I.W. (2000). Mol. Cell *5*, 1013–1024.

Jin, T., Zhang, N., Long, Y., Parent, C.A., and Devreotes, P.N. (2000). Science *287*, 1034–1036.

Kalab, P., Weis, K., and Heald, R. (2002). Science *295*, 2452–2456.

Karsenti, E., and Vernos, I. (2001). Science *294*, 543–547.

Kraynov, V.S., Chamberlain, C., Bokoch, G.M., Schwartz, M.A., Slabaugh, S., and Hahn, K.M. (2000). Science *290*, 333–337.

Lauffenburger, D.A., and Horwitz, A.F. (1996). Cell *84*, 359–369.

Smith, A.E., Slepchenko, B.M., Schaff, J.C., Loew, L.M., and Macara, I.G. (2002). Science *295*, 488–491.